Induction of Urogenital Anomalies and Some Tumors in the Progeny of Mice Receiving Diethylstilbestrol during Pregnancy

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SUMMARY

Pregnant mice were given a single dose (10 μg/g body weight) of diethylstilbestrol (DES) on Days 7 to 19, which correspond to the first to fifth lunar months in humans, after the authors, using a 14C-labeled compound, confirmed easy placental penetration by DES. Treatment with DES on Days 15 to 19 resulted in the induction of persistent urogenital sinus (15.8 to 92.5%) and hypertrophy of the portio vaginalis (11.8 to 73.3%) in female offspring, and treatment on Days 17 and 19 resulted in the induction of undescended testes (11.8 to 73.3%) in male offspring, although treatment with DES at other stages of pregnancy and after birth did not cause these alterations. The incidence of various tumors (lung adenoma, granulosa cell tumor, etc.) increased significantly (31.0 to 37.9%) when DES was given on Days 15 and 17, which correspond to the stage sensitive to other carcinogens. However, adenosis and adenocarcinoma of the vagina were not observed in the offspring.

INTRODUCTION

In 1971, Herbst et al. (15) reported that synthetic stilbestrol (DES) therapy for abortion during pregnancy induced vaginal cancer in resulting children 14 to 22 years later. Since the 1st 8 cases were described, several others have been reported (13, 14, 39). Fortunately, the association of adenocarcinoma of the vagina with maternal stilbestrol therapy was discovered easily, as was the case in the thalidomide disaster, because this tumor is extremely rare so early in life. In experimental animals, transplacental toxicities, including carcinogenesis with chemicals, have been reported before the use of DES (19, 34). However, no significant carcinogenicity of DES was detected in newborn mice (8-9), although adenotic changes were observed in the uterine cervix and vagina after neonatal treatment with DES (7, 8) and estrogen (7) and although anterior pituitary tumors and adenosis were not observed in newborns also were treated with DES because they are considered to be sensitive to estrogens (3, 4, 7, 8, 17, 37).

MATERIALS AND METHODS

Animals. ICR/Jcl virgin female mice (Japan Central Laboratory for Experimental Animals, Tokyo, Japan) were used. An estrous female was placed in a cage with a breeder male at 10 p.m., and next morning a vaginal plug was checked to determine Day 1 of gestation (27). Animals were maintained on Mouse Diet CA-1 (CLEA Japan, Tokyo, Japan) (23).

Comparison of the Time Period of Embryonic Ages of Human and Mouse. The time period of embryonic and fetal development in humans and ICR/Jcl mice was compared using previous data on humans (5, 18) and serial sections of Day 5 to Day 19 embryos and fetuses of this strain of mouse. Day 5 of ICR/Jcl mouse embryo corresponds to the 6th day (ovulation age) of human embryos, Day 7 to the 9th, Day 9 to the 19th to 20th, Day 11 to the 28th to 30th, Day 13 to the 39th to 48th, Day 15 to the 50th to 60th, Day 17 to the 80th to 95th, and Day 19 to the 94th to 115th day. These time periods are approximately the same as those reported previously for other strains of mice (30, 31). Human ages are approximate and may be off by 10 days or more in the later stages (5, 18). Ovulation age means the embryonic age after ovulation and corresponds to the obstetric age (which is calculated from the onset day of the last menstruation and is usually shown in lunar months) minus 14 days.

Radioactivity Measurement. One μCi of [monoethyl-1-14C]DES (specific activity, 52.0 mCi/7mmole; The Radiochemical Centre, Amersham, England) per g body weight was injected s.c. into the Day 18 pregnant mice. The pregnant mice were paired and killed 1 and 5 hr after injection, and 10 to 15 mg of specimens of various organs from each pair and their fetuses were solubilized in 1.0 ml of Soluent 100 (Packard Instrument Co., Inc., Downer’s Grove, Ill.). Fifteen ml of scintillator was added into solubilized tissues, and the total 14C was measured by liquid scintillation counter (29).
Treatment during Pregnancy. Pregnant mice received a single s.c. injection of 10 \( \mu g \) of DES disodium salt per g body weight (Tokyo Kasei Co., Tokyo, Japan) dissolved in water on Day 7, 9, 11, 13, 15, 17, or 19. For exclusion of a milk factor, the offspring exposed to DES on Day 19 were foster-nursed by untreated lactating mothers and the offspring receiving water on Day 19 were foster-nursed by the Day 19 DES-treated mothers. As a natural estrogen, 20 \( \mu g \) of 17\( \beta \)-estradiol (provided by Dr. S. S. Koide, The Rockefeller University, New York, N. Y.) per g were given on Day 19. The offspring were separated from the mothers 4 weeks after birth and were killed the 12th month after birth. The dose of DES corresponds to the maximum dose tolerated by pregnant ICR/Jcl mice; all 13 pregnant mice receiving larger amounts of DES (20 to 500 \( \mu g/g \)) aborted because of abruption of the placenta. A high dose of DES (500 \( \mu g/g \)) was given to 8 pregnant mice on Day 10, and external malformations of their fetuses were examined by hysteroscopy immediately after the discovery of vaginal bleeding (Days 14 to 15). All of the fetuses isolated just before abortion were still living in utero.

Treatment of Neonates and Young Mice. Male and female neonates received a single s.c. injection of 50 \( \mu g \) of DES per g within 12 hr after birth. Thirty young male and 30 young female mice (21 days old and weighing 9 to 11 g) received a single s.c. injection of DES at a dose of 10 \( \mu g/g \) and 20 young male and 20 young female mice received DES at a dose of 100 \( \mu g/g \). These mice were killed 12 months after treatment. Internal organs were carefully examined for gross pathological lesions, especially tumors and malformations, and were fixed in 10% formaldehyde solution. Specimens stained with hematoxylin and eosin were microscopically examined.

RESULTS

Distribution of Radioactivity. \( ^{14}C \) was detected in the various organs of the fetus at about 50% of maternal concentration 1 hr after injection of \( ^{14}C \)-labeled DES into the Day 18 pregnant mice, and the radioactivity remained in higher concentration in the fetus than in the mother 5 hr after injection (Table 1).

Urogenital Anomalies. Treatment with a high dose of DES (500 \( \mu g/g \) on Day 10 induced the abruption of the placenta, but malformations were not observed in the fetuses. The incidence (0 of 99, 0.0%) was not different \( (p = 1.0) \) from that of controls receiving water (1 of 320, 0.3%). Treatment with a lower dose of DES (10 \( \mu g/g \) on Day 17 resulted in a high frequency of abortion and a decrease in the average number of live births, but abortion was not observed at other stages (Table 2). At birth, no anomalies were observed macroscopically in the offspring. Eight to 9 days after birth, opening of the anterior portion of the vaginal orifice, which is closed until 4 weeks of age in normal development, was observed in the female offspring of mice treated on Days 15 to 19 (Chart 1). This finding became clearer after 21 days of age. It is easy to detect this urogenital anomaly, because urine flows from the vagina when the lower abdomen is pressed. Anatomically, the urethra opens into the vagina (persistent urogenital sinus) (Fig. 1). The same anomaly was induced in a significant number of mice with estradiol (Table 2). When male offspring were killed the 12th month after birth, undescended testes and their hypogenesis were observed in a significant number of the male offspring of mice treated on Days 17 and 19 (Table 2). Undescended testes were small (50 to 90 mg) when compared with those of controls [133.8 \( \pm \) 2.6 (S.E.) mg] and spermatogenesis was not observed in some typical hypogenetic testes. Histological patterns were reported previously by Nomura (25). These offspring were sterile. These urogenital anomalies in male and female mice were not observed when DES was given at other stages of gestation or after birth.

Tumors. The incidence of tumor-bearing mice per survivor was low but significantly higher in the offspring of mice receiving DES on Days 15 and 17 than in controls (Table 3). The incidence of ovarian tumors (cystadenomas and granulosa cell tumors) was also significantly higher in these offspring than in controls. The incidence of lung tumor (papillary adenoma) was significant in offspring of mice receiving DES on Day 15. Ovarian tumors were also induced in significant numbers in mice receiving an extremely high dose of DES (100 \( \mu g/g \)) at a young age (Table 3). However, adenosis and adenocarcinoma in the vagina were not observed in the offspring.

Other Lesions in Urogenital and Reproductive Organs. Some female offspring with urogenital anomalies developed large, firm, tumorous masses on the posterior wall of the vagina approximately 6 weeks after birth (Fig. 2). However, they were not adenocarcinomas but were composed of granulomas and proliferating sebaceous glands. Treatment on Days 15 to 19 also induced hypertrophy of the portio vaginalis. Ovaries persisting with no oogenesis were

<table>
<thead>
<tr>
<th>Organs</th>
<th>1 hr</th>
<th>5 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>181.6 (1.00)*</td>
<td>29.5 (1.00)</td>
</tr>
<tr>
<td>Lung</td>
<td>414.1 (2.28)</td>
<td>75.9 (2.58)</td>
</tr>
<tr>
<td>Liver</td>
<td>2640.7 (14.10)</td>
<td>859.9 (29.00)</td>
</tr>
<tr>
<td>Brain</td>
<td>202.5 (1.11)</td>
<td>30.7 (1.03)</td>
</tr>
<tr>
<td>Uterus</td>
<td>156.5 (0.86)</td>
<td>44.3 (1.49)</td>
</tr>
<tr>
<td>Vagina</td>
<td>117.1 (0.64)</td>
<td>44.6 (1.50)</td>
</tr>
<tr>
<td>Ovary</td>
<td>75.0 (2.53)</td>
<td></td>
</tr>
<tr>
<td>Placenta</td>
<td>162.0 (0.89)</td>
<td>75.9 (2.56)</td>
</tr>
<tr>
<td>Uterus*</td>
<td>81.2 (0.45)</td>
<td>79.1 (2.68)</td>
</tr>
<tr>
<td>Amnion</td>
<td>40.0 (0.22)</td>
<td>59.3 (2.00)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, ratio of radioactivity in each organ to that in maternal blood.

† In the case of the fetal uterus, uteri were collected from all female fetuses of 2 pregnant mice, and the weight and radioactivity were measured.

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Table 2
Urogenital anomalies and hypertrophy of the portio vaginalis in mice treated with DES at fetal, neonatal, and young ages

Incidence of urogenital anomaly in female offspring shows the malformation-bearing offspring per survivor the 4th week after birth or treatment. Testicular anomaly, hypertrophy of the vagina, and other anomalies were recorded at the time of sacrifice. The χ² test was applied with Yates's correction.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of pregnant mice</th>
<th>No. of live at 4 wk</th>
<th>Urogenital anomaly in females</th>
<th>Testicular anomaly</th>
<th>Hypertrophy of portio vaginalis</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>DES</td>
<td>Day 7</td>
<td>10</td>
<td>2 (1)*</td>
<td>11</td>
<td>11.0</td>
<td>6</td>
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<tr>
<td>DES</td>
<td>Day 9</td>
<td>10</td>
<td>4 (4)</td>
<td>40</td>
<td>10.0</td>
<td>17</td>
</tr>
<tr>
<td>DES</td>
<td>Day 11</td>
<td>10</td>
<td>5 (4)</td>
<td>42</td>
<td>10.5</td>
<td>23</td>
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<tr>
<td>DES</td>
<td>Day 13</td>
<td>10</td>
<td>4 (3)</td>
<td>30</td>
<td>10.0</td>
<td>11</td>
</tr>
<tr>
<td>DES</td>
<td>Day 15</td>
<td>10</td>
<td>5 (4)</td>
<td>35</td>
<td>8.8</td>
<td>15</td>
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<tr>
<td>DES</td>
<td>Day 17</td>
<td>10</td>
<td>13 (7)</td>
<td>49</td>
<td>7.0</td>
<td>12</td>
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<tr>
<td>DES</td>
<td>Day 19</td>
<td>10</td>
<td>12 (12)</td>
<td>110</td>
<td>9.2</td>
<td>40</td>
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<tr>
<td>Water</td>
<td>Day 19</td>
<td>0</td>
<td>2 (2)</td>
<td>20</td>
<td>10.0</td>
<td>5</td>
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<tr>
<td>Estradiol</td>
<td>Day 19</td>
<td>20</td>
<td>5 (4)</td>
<td>35</td>
<td>8.8</td>
<td>14</td>
</tr>
<tr>
<td>DES</td>
<td>Newborn</td>
<td>50</td>
<td>6 (6)</td>
<td>64</td>
<td>10.7</td>
<td>9</td>
</tr>
<tr>
<td>DES</td>
<td>21 days</td>
<td>10</td>
<td>30 (30)</td>
<td>0/30</td>
<td>0.0</td>
<td>0/18</td>
</tr>
<tr>
<td>DES</td>
<td>21 days</td>
<td>100</td>
<td>30</td>
<td>0/30</td>
<td>0.0</td>
<td>0/18</td>
</tr>
<tr>
<td>DES</td>
<td>Mother</td>
<td>10</td>
<td>20</td>
<td>0/17</td>
<td>0.0</td>
<td>0/17</td>
</tr>
<tr>
<td>Estradiol</td>
<td>Mother</td>
<td>20</td>
<td>30</td>
<td>0/30</td>
<td>0.0</td>
<td>0/17</td>
</tr>
<tr>
<td>Control</td>
<td>25 (25)</td>
<td>260</td>
<td>10.4</td>
<td>46/77</td>
<td>77/77</td>
<td>0/77</td>
</tr>
</tbody>
</table>

* Number of mice that delivered live offspring. The others resulted in abortion.
* NS, not significant; HT, hydrops testis; O, obesity (more than 60 g); E, edema.
* Number of mice that developed a tumorous mass in the vagina.
* Mother mice were exchanged immediately after delivery. Details are given in the text.
* Number of mice used in this experiment and sacrificed the 14th month after birth. Controls were untreated.
complicated with ovarian tumors (contralateral ovaries), urogenital anomalies, and hypertrophy of the portio vaginals. Histological patterns of these ovaries were the same as those exposed to urethan during pregnancy (25). These female offspring were sterile.

DISCUSSION

Detection of high \(^4\text{C}\) radioactivity in the various organs of fetuses receiving \([\text{\textsuperscript{14}C}]\text{DES}\) indicates that DES or its metabolites can pass through the mouse placenta easily, but not as easily as urethan can (29), when compared with other chemical carcinogens (approximately 1%) (1, 2, 36, 38). Specific accumulation of \(^4\text{C}\) in the urogenital organs of the fetus was not observed macroautoradiographically (data not shown). The transferred compounds induced urogenital anomalies and some tumors in the male and female offspring. However, the patterns of teratogenicity were extremely different from that for other nonhormonal agents (21, 23, 26, 28, 32). Treatment with DES on Day 10, which corresponds to the time of differentiation of various organs and to the stage most sensitive to induction of various kinds of malformations with various chemicals in this strain of mice (23, 26, 28), did not induce malformations even if very large amounts of DES were given. However, treatment with a lower dose of DES on Days 15 to 19 induced urogenital anomalies in the offspring. This is because Days 15 to 19 (the 3rd to 5th lunar months in humans) correspond to the stage of differentiation of the urethra and vagina from the urogenital sinus, a process controlled by androgens secreted from the fetal testes (16). However, urogenital anomalies have not been reported in humans receiving DES during the fetal stage. The difference may be due to the pattern of exposure to DES. The mouse received a single injection of 10 \(\mu\)g of DES per g, while the dose for humans was approximately 0.1 to 2 \(\mu\)g/g/day for more than 3 months (13). In the case of chemical teratogenesis, there exists a sharp threshold dose and a critical stage (29).
DES induced low but significant yields of lung and ovarian tumors in the offspring when given on Days 15 and/or 17, which is the stage of the ICR/Jcl mouse fetus most sensitive to tumor induction with various chemical carcinogens (23–25, 27, 28). A high dose of DES also induced ovarian tumors when given to young mice. These tumor incidences would indicate that DES has a nonhormone-like carcinogenic action on the lung cells, which are not the target of estrogen. Estrogens usually induce tumors in hormone-related organs, such as the pituitary gland, the mammary gland, and the uterus (3, 4, 9–11). Also DES may have increased tumor susceptibility by damaging immunosurveillance (35), because the lungs and ovaries of ICR/Jcl mice are known to be sensitive to various carcinogens for tumor induction (22–25, 27–29). However, a single injection of DES did not induce vaginal adenocarcinoma in mice, although it was given during the stages of the developing mouse embryo and fetus (Days 7 to 19) that correspond to the stages most sensitive to DES in human fetuses (9 to 115 ovulation ages, i.e., 1st to 5th lunar months) for the induction of vaginal cancer (13–15). It might be necessary to give smaller amounts of DES continuously during pregnancy, as happens in humans.

Furthermore, the failure to produce vaginal adenocarcinomas in mice in this study and in previous studies with newborn mice (3, 4, 6–8, 17, 37) may simply reflect the fact that most carcinogens only increase the incidence of tumors that occur spontaneously (20), as do lung and ovarian tumors in ICR/Jcl mice (25). Spontaneous cancers of the vagina are rare in mice (no report for adenocarcinoma), as they are in all animals studied other than humans (20). Dunn and Green (6) observed a few vaginal tumors (3 of 12 in BALB/c and 1 of 10 in C3Hf) 18 to 26 months after neonatal treatment with DES, but the incidence was not significantly different from controls (1 of 4 in C3Hf) and the induced tumors were not adenocarcinomas.

Adenosis in the cervix and vagina after neonatal treatment with estrogen (7) and DES (7, 8) has also been reported in mice by several investigators, but it was dominant in the cervix and had not transformed to adenocarcinoma (8). Prenatal treatment in this study did not induce adenosis in the vagina and cervix, but instead induced a tumor mass (proliferating sebaceous gland) in the vagina and masculinizations, such as persistent urogenital sinus, hypertrophy of the portio, and ovarian hypogonadism. Hypogynic ovary is considered to be precancerous (20, 25). Masculinization of other external genital organs, such as clitoromegaly, by very large amounts of estrogens (12) and by androgenic hormones (33) was also reported in rats. The androgenic action of large amounts of DES and estradiol remains to be elucidated. Furthermore, neonatal treatment with DES did not induce vaginal adenosis in this experiment. This may be due to the fact that extremely high doses (50 μg/g), 10 to 20 times the dose used in the previous work (8), killed affected newborns (Table 2). Although tumor masses were not transplantable even if observed more than 2 years after birth, further study on this vaginal lesion remains to be done with different strains and species of experimental animals, because the onset of this lesion is the same as that in humans (at puberty).

ACKNOWLEDGMENTS

We thank Dr. S. S. Koide for providing radioisotope and for his advice; Dr. Y. Sakamoto, Dr. T. Higashi, and Dr. N. Tateishi for their advice and help; Dr. J. F. Crow, Dr. J. A. Miller, Dr. E. C. Miller, Dr. K. Matsumoto, and Dr. H. A. Bern for their advice in preparing the manuscript; and W. Kugler, Jr., for taking the photographs.

REFERENCES


Fig. 1. Female offspring with persistent urogenital sinus and portio vaginalis hypertrophy (left) and mother with normal vaginal wall and endometrial hyperplasia of the uterus. Vagina was cut sagittally at the posterior wall. a, urethral mucosa; b, hypertrophy of the portio vaginalis; c, uterus; d, normal vaginal wall; e, endometrial hyperplasia.

Fig. 2. Sagittal section of tumorous mass in the vagina. a, tumorous mass; b, hypertrophy of the portio vaginalis; c, rectum.
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